# THE EFFECTS OF DIMETHYL SULFOXIDE ON THE MUTAGENIC ACTION OF ETHYL METHANESULFONATE IN DROSOPHILA MELANOGASTER

An abstract of a Thesis by
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The problem. Ethyl methanesulfonate (EMS) is a potent alkylating agent, capable of inducing mutations in Drosophila at frequencies as great as 70%. EMS is often referred to as a radiomimetic substance because of the similarities between the clinical manifestations of radiation damage and injury due to alkylating agents. Dimethyl sulfoxide (DMSO) provides an effective protection against the mutagenic effects of radiation in many organisms including Drosophila. The major objective of this study was to determine the effects that topical application of DMSO would have on the mutagenic action of ingested EMS.

Procedure. Adult male Drosophila were treated in four ways. The five separate groups tested were treated with DMSO alone, EMS alone, DMSO followed by EMS, and EMS followed by DMSO. The fifth group was a control and received no treatment. Treated adult males were mated individually to three or four virgin Basc females. The Basc technique for detection of X-linked recessive lethal mutations was followed. Mutation frequencies were computed and subsequently submitted to chi square analysis.

Findings. The control group produced a mutation frequency of 0.0%. This was used as a spontaneous X-linked recessive lethal mutation rate. The DMSO group demonstrated a 1.3% mutation rate. The EMS group produced a 35.9% mutation frequency. Pre-treatment with DMSO followed by EMS treatment resulted in a mutation rate of 31.6%. DMSO treatment following EMS treatment resulted in a 35.0% mutation frequency.

Conclusions. The data obtained from this study indicates that DMSO does not act as an antimutagenic substance against EMS in Drosophila. DMSO does appear to be slightly mutagenic after topical application and this application seems to reduce the overall reproductive capabilities of the treated males.

Recommendations. Further experimentation with various concentrations of DMSO and different methods of administration is necessary to fully elucidate the actual effects of DMSO in Drosophila.

# THE EFFECTS OF DIMETHYL SULFOXIDE ON THE MUTAGENIC ACTION OF ETHYL METHANESULFONATE IN *DROSOPHILA MELANOGASTER*

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by

Paul Frederick Kopeck

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# INTRODUCTION AND REVIEW OF THE LITERATURE

The ability to alter the genetic constitution of a cell is a characteristic which has been attributed to a variety of physical phenomena and chemical compounds. Muller (1927) reported that X-rays could induce gene mutations and rearrangements in Drosophila. Stadler (1928) confirmed the findings of Muller by demonstrating that X-rays could also induce mutations in barley. Following these discoveries, the ability of ultraviolet light to induce point mutations in Drosophila was observed by Altenburg (1930). Later, Auerbach and Robson (1946) established that certain chemical compounds (sulfur and nitrogen mustards), which produced clinical manifestations similar to those resulting from X-ray damage, could also induce mutational lesions. Today, it is believed that there are over 10,000 natural and synthetic chemical agents in the environment capable of producing irreversible genetic damage in man (Fishbein, Flamm, and Falk, 1970).

The significant effect that the mutational process exerts on man has initiated extensive research in an attempt to provide a detailed understanding of the mechanical aspects of genetic alteration. These investigations have lead not only to the elucidation of the mutagenic process, but have frequently provided an understanding of other genetic phenomena such as the process of gene regulation and

the translation of the genetic code (Drake, 1970).

One major area in the study of mutagenesis is the investigation of antimutagenic substances. A number of antimutagenic substances have been reported in the literature. Puglisi (1968) observed that both actinomycin D and basic fuchsine are strongly antimutagenic in Saccharomyces cerevisiae. Grigg and Stuckey (1966) demonstrated that caffeine, a known mutagen, could also act as an antimutagen for Escherichia coli. Spermine has been reported as an effective antimutagen for multiplying E. coli and Staphylococcus aureus (Johnson and Bach, 1965). Protection against radiation induced mutations has been afforded by a variety of compounds such as cysteine (Patt et al., 1949), S(2-aminoethyl)isothiuronium bromide hydrobromide (Doherty and Burnett, 1955), and dimethyl sulfoxide (DMSO) (Ashwood-Smith, 1961).

The purpose of this study was to determine the effect that DMSO, a radioprotective substance might have on the mutagenic action of the chemical mutagen ethyl methanesulfonate, a potent alkylating agent.

## Ethyl Methanesulfonate (EMS)

EMS ($CH_3CH_2OSO_2CH_3$) is a monofunctional alkylating agent. All alkylating agents are separated into two broad categories, the monofunctional and the bi- or polyfunctional alkylating agents. These two categories are based on the

ability of the compound in question to form one, or two or more reactive alkyl groups (Fishbein et al., 1970). The major reactive group on the EMS molecule is the ethyl group and this compound carries out its mutagenic action through the ethylation of nuclear DNA (Sega, Gee, and Lee, 1972).

Most, and probably all of the alkylating agents have some mutagenic effect, regardless of the category to which they belong. These compounds induce point mutations (mainly transitions), chromosome breaks, and chromosome mutations. The presence of only one reactive alkyl group within the alkylating compound is sufficient for the production of a mutation (Hollaender, 1971).

EMS is an extremely potent chemical mutagen which has been shown to induce both mosaic and complete lethal mutations in the mature sperm of Drosophila (Fahmy and Fahmy, 1957; Alderson, 1965; Epler, 1966; Jenkins, 1967; Kahn, 1969). Feeding adult male Drosophila 0.0161M EMS for twenty-four hours resulted in a complete X-linked recessive lethal mutation rate of 18.3% (Fahmy and Fahmy, 1957). Alderson (1965) fed adult male Drosophila a 0.0195M concentration of EMS to produce a complete X-linked recessive lethal mutation frequency of 41.5%. In the same experiment a 0.0132M concentration of EMS produced a complete X-linked recessive lethal mutation rate of 48.0%. Kahn (1969) has demonstrated that feeding adult male Drosophila 0.012M EMS for twenty-four hours resulted in a complete X-linked recessive lethal

mutation rate of 43.6% when the treated sperm were used immediately. If the sperm were stored in the female for six days, a complete X-linked recessive lethal mutation rate of 38.7% was observed. This data suggests that the storage of treated sperm in the female has no significant effect on the production of complete X-linked recessive lethal mutations following treatment of male Drosophila.

In contrast to the feeding technique of the previous workers, Epler (1966) used an injection method to administer the EMS. After injection of 0.5 µl of 0.04M EMS into the abdomen of Oregon-R males, a complete X-linked recessive lethal mutation rate of 27.65% was observed. In the same experiment, injection of 0.5 µl of 0.02M EMS resulted in the production of a 12.36% mosaic X-linked recessive lethal mutation frequency, and a 15.83% complete X-linked recessive lethal mutation frequency.

With yet a different technique, Sega and Lee (1970) have observed a complete X-linked recessive lethal mutation rate of 42.0% after treating adult male Drosophila with 10.0 µl of 0.12M EMS for one hour by means of a vacuum injection method using EMS vapor. This new technique, devised by Sega and Lee (1970) and improved by Sega and Cumming (1973) is the least variable method of administering EMS that has yet been used. With this method, ten nonetherized adult males are placed in a 25 ml serum vial. The absolute pressure in the vial is lowered to between 40 and 50 mm of Hg.

10 µl of the mutagen dissolved in water is then introduced into the vial with a 1.0 ml syringe. Atmospheric pressure forces the mixture out of the syringe into the vial as an aerosol. The flies are allowed to remain in the vacuum one to two hours as the mutagen is absorbed by the flies through their tracheae.

It is apparent from the mutation rates observed by the investigators mentioned above that feeding EMS and the vacuum injection technique of administering EMS are the most efficient means of treating Drosophila with this mutagen. The microinjection method of administering EMS is less effective because the amount of EMS that actually remains in the abdomen after injection is extremely variable (Carlson and Oster, 1962).

In the production of germinal mutations, EMS has its greatest effect on the late stages of spermatogenesis. Analysis of the brood patterns of EMS-induced mutations in Drosophila demonstrate that this alkylating agent is most effective in the latest stages of meiosis including the spermatid and spermatozoa stages. It has been shown that sperm ejaculated in the first seven days after treatment had been derived from post-meiotic germ cells (spermatids and spermatozoa) at the time of treatment (Martin, 1965). These spermatozoa produced a much higher frequency of complete X-linked recessive lethal mutations than did the spermatozoa that were in meiotic or pre-meiotic stages at the time of

treatment. The ratio of post-meiotic or pre-meiotic induced lethals was 28.4:1 (Fahmy and Fahmy, 1957).

The mechanism of action of EMS at the molecular level is disputed at the present time. Brookes and Lawley (1961), using acid hydrolysis, showed that the most frequent site of attack by EMS in DNA was at the N-7 position of guanine. It is the ethyl group of the EMS molecule which attacks the N-7 position of the guanine residue. It is not unexpected that the N-7 position of guanine appears to be the site of the greatest amount of action by EMS. Wave-mechanical studies and three-dimensional model building have shown that the N-7 position of guanine is sterically available by being positioned in the wide groove of the DNA double helix (Fishbein et al., 1970). Lawley and Brookes (1963) suggest that EMS may also attack the N-3 position of adenine, the N-1 position of adenine, and the N-1 position of cytosine. Fishbein and his associates (1970) agree that these positions of the various nitrogenous bases are sites of ethylation by EMS, but state that these reactive sites are attacked at a much lower frequency by EMS than either the N-7 or the N-3 position of guanine.

The preferential alkylation of guanine that can be demonstrated *in vitro* also appears to hold true *in vivo* (Auerbach, 1967). The alkylated guanine is capable of inducing a mutation by erroneously pairing with thymine

instead of cytosine. This incorrect pairing results in a transition mutation in which an adenine-thymine base pair has replaced a cytosine-guanine base pair (Lawley and Brookes, 1961).

In addition to transitions, alkylating agents have also been observed to produce transversions. To explain this end result, Freese (1961) proposes that the alkylated guanine residue is capable of dissociating itself from the DNA sugar-phosphate backbone. This depurination results in an empty slot in the sequence of bases along one strand of the DNA double helix. The empty position is referred to as an "apurinic gap" (Bautz and Freese, 1960). This gap may be filled with a purine other than guanine to produce a transition mutation, or it may be filled with a pyrimidine, in which case a transversion mutation occurs. If the gap is not repaired in some manner, a deletion will result (Freese, 1961).

More recently, Sega, Gee, and Lee (1972) have devised a method for determining the quantity of ethylation that has taken place in the sperm cells of Drosophila after treatment with EMS. The number of ethylations per sperm cell DNA was found to range from $1.0 \times 10^{7}$ to $3.0 \times 10^{8}$. By feeding 0.025M EMS to Drosophila for twenty-four hours it was demonstrated that from 3% to 100% of the nucleotides in the sperm cell DNA could be ethylated, assuming one ethylation per nucleotide. Combining these results with the number of complete

X-linked recessive lethal mutations produced by feeding EMS to Drosophila in similar concentrations, indicates that roughly, only one X-linked recessive lethal mutation occurs for every $10^7$ ethylations of the X chromosome euchromatin. The authors believe that the observed mutations induced by EMS could not be due to ethylation of a common nucleophilic site on DNA followed by a constant probability of base mispairing at each DNA duplication. They suggest that the mutagenic action of EMS in Drosophila is the result of some rare chemical event or a unique combination of ethylations, either within the same nucleotide or in several neighboring nucleotides. Such a site, which is only rarely attacked by EMS, could be the 0-6 position of guanine (Loveless, 1966).

Auerbach (1967) first observed the effects of a chemical mutagen when she tested the mutagenicity of mustard gas, an alkylating agent, in Drosophila. She chose to test this chemical because of the clinical similarities between mustard gas burns and X-ray burns, coupled with the knowledge that X-rays were capable of causing damage to chromosomes and genes. The abnormalities of chromosome structure typically produced by ionizing radiation and many of the alkylating agents involve aberrations in the linear ordering of the genetic material (Loveless, 1966). This would include mispairing of bases to produce transitions and transversions in addition to deletions that lead to frame-shift mutations. The similarity of the effects of irradiation and alkylating

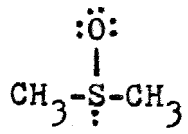
agents has earned many of the alkylating agents the classification as "radiomimetic" substances. All of the types of chromatid aberrations produced by radiations have also been seen following treatment with alkylating agents (Loveless, 1966). These and other observations demonstrated that the alkylating agents often did produce mutations similar to those produced by radiations, therefore living up to their classification as "radiomimetic" substances. However, it was eventually brought to light that the relationship between these two mutagens was not as simple as it appeared.

Although the actions of alkylating agents are similar to the mutagenic actions of radiations in many ways, Loveless (1966) has compiled a list of differences between the two. The list includes the following: (1) The number of rearrangements relative to X-linked recessive lethals is considerably smaller with alkylating agents than with radiations. (2) Alkylating agents induce a higher percentage of mosaics. Drosophila with mosaic gonads transmit this mosaicism to their offspring. This is very rarely seen following irradiation. (3) Alkylating agents are indifferent to the presence or absence of oxygen during treatment; the yield of aberrations induced by X-rays is higher in the presence of oxygen than in its absence. It can be interpreted from this listing that although alkylating agents and irradiations have many similarities, there are also exceptions to statement that alkylating agents can be referred to as radiomimetic

substances.

Dimethyl Sulfoxide (DMSO)

DMSO ($CH_3SOCH_3$) is a dipolar, aprotic, hydroscopic solvent (MacGregor, 1967). Structurally, the DMSO molecule appears pyramidal in shape (Rammler, 1971).



This molecule possesses a marked polarity due to the appreciable separation of the sulfur and oxygen atoms. This polarity is responsible for the high dielectric constant of DMSO in the liquid form and may also be responsible for some of its unique solvent properties (David, 1972).

DMSO is completely miscible with water, this action taking place with the evolution of some heat. It has been demonstrated that DMSO acts as an acceptor atom in hydrogen bonding and that between DMSO and water this activity is 1.33 times as strong as between water-water (MacGregor, 1967). Rammler and Zaffaroni (1967) suggest that a 2:1 association complex is formed between water and DMSO. In addition, DMSO is a better solvent than water for many substances (Szmant, 1971). David (1972) states that the ability of DMSO to penetrate skin and mucous membranes may be the result of its dynamic interaction with tissue water. This would be accomplished through the formation of

hydrophobic bonds between DMSO and exposed moieties (N-H and O-H) of the protein structure. The alteration in the configuration of the protein structure brought about by DMSO interaction may explain the penetration of DMSO through the skin. These alterations in the immobile protein structures take place rapidly and are reversible.

Although DMSO may be best known for its ability to rapidly penetrate the intact dermis (Herschler and Jacob, 1965) and mucous membranes (Jacob, Bischel, and Herschler, 1964) and to function as a carrier for a variety of substances (Horita and Weber, 1964), this compound possesses a number of other significant biological properties. Jacob and his associates (1964) list a number of primary pharmacologic effects of DMSO in some higher organisms including man. This list includes analgesia, anti-inflammatory activity, antipyretic activity, diuresis, vasodilation, cholinesterase inhibition, smooth muscle stimulation, and bacteriostasis. In addition to these activities, DMSO has been proven to be an effective cryoprotective agent (Lovelock and Bishop, 1959) and a radioprotective substance (Ashwood-Smith, 1961) for tissues and cells.

The radioprotective activity of DMSO was first demonstrated by Ashwood-Smith (1961). He observed that intraperitoneal injection of 4,500 mg DMSO/kg live weight in mice up to one hour before administration of a lethal dose of X-rays produced a 70% survival rate. Increasing the dosage

of DMSO above 4,500 mg/kg had no increased effect on the survival rate. Injection of the same amount of DMSO more than one hour before irradiation reduced the effectiveness of the compound. Injection four hours before irradiation resulted in no positive effect. After irradiation, injection of DMSO had no effect on mortality. After determining how much radiation was needed to produce an $LD_{50}$ following treatment with DMSO, a dose reduction factor (DRF) of 1.33 was computed for DMSO in mice. The $LD_{50}$ is 825 rads and this was increased to approximately 1,100 rads after DMSO treatment. Ashwood-Smith concluded that the radioprotective action of DMSO was related to the absolute concentration of sulfoxide present in the tissues during irradiation in that the immediate metabolic products of DMSO did not protect against irradiation.

DMSO protection against the action of ionizing radiations has now been established for a number of organisms and subcellular systems. Bridges (1962a) demonstrated a considerable radioprotective effect by DMSO in some Pseudomonas species at concentrations of 0.5 and 1.0M. Radioprotective action of DMSO for mice has been reported by other investigators (Moos and Kim, 1966; Moos, LeVan, and Mason, 1967; Gollan, 1967; Hagemann, Schenken and Evans, 1969) in addition to the work of Ashwood-Smith (1961). Highman, Hansell, and White (1967) demonstrated a radioprotective effect of DMSO in rats. The effect of DMSO on the radiation response of the

rough skinned newt (Taricha granulosa) has been reported by Lappenbusch and Willis (1970). Soaking seeds in 10% DMSO for four hours before irradiation produced a highly significant radioprotective effect in a variety of higher plants such as barley, wheat, and Triticale (Kaul, 1970).

In addition to these single and multicellular organisms, DMSO has been observed to produce radiation protection in some subcellular systems. Vos and Kaalen (1962) have demonstrated a radioprotective action by DMSO, at a number of concentrations, in an established line of human kidney cells perpetuated in tissue culture. The radioprotective influence of DMSO on the radiation sensitivity of rabbit catalase has been reported (Lohmann, Moss, and Perkins, 1965), and Lohmann, Moss, and Barker (1966) have observed a radioprotective effect exhibited in a variety of DMSO concentrations in rabbit muscle lactate dehydrogenase.

Alexander (1966) reported the effects of DMSO treatment of Drosophila. She administered DMSO to Drosophila through methods of dipping, injection, and feeding. Her results indicated that the various germ cells of the spermatogenic cycle showed no radioprotection from DMSO when treatments were made by injection, feeding, or dipping four hours before radiation treatment.

More recently, Mazar Barnett (1972) has demonstrated that DMSO does provide radioprotection to the living organism and to the germ cells in Drosophila melanogaster. Without

DMSO treatment the complete X-linked recessive lethal mutation frequency was 2.78% for the first 3.30 hours of the mating period with 1,000r of X-rays and 1.51% for the remainder of the twenty-four hour mating period after irradiation. This mutation frequency was reduced to .88% and 1.04% respectively when intraabdominal injection of 10% DMSO in 0.4% NaCl was made thirty minutes before irradiation with 1,000r of X-rays. Injection of 10% DMSO twenty hours before irradiation also reduced the frequency of complete X-linked recessive lethal mutations from 2.78% to 1.20% and 1.51% to 1.42% for the two mating periods mentioned above. These results establish the fact that DMSO can act as a radioprotective agent in Drosophila.

The radioprotective effect of DMSO has been attributed to a variety of mechanisms since it was initially observed by Ashwood-Smith (1961). Bridges (1962b) stated that he believed that the sulfur atom of DMSO played a major role in the mechanism of radioprotection. Van der Meer, Valkenburg, and Remmelts (1963) reported the protective action of DMSO in two strains of mice. They observed that radioprotective dose of DMSO resulted in a marked reduction of the oxygen tension in the spleen. A similar effect had been described for a number of other radioprotective substances such as histamine and epinephrine (Van der Meer and Van Bekkum, 1959), tryptamine (Van der Meer and Van Bekkum, 1961), and cyanide (Van der Meer and Valkenburg, 1961). From these observations,

it was suggested that DMSO protects in the mouse by causing hypoxia in the spleen and possibly other blood forming organs.

Highman, Hansell, and White (1967) demonstrated that DMSO was capable of radioprotection in rats. In the discussion of their results they cite the work of Vos and Kaalen (1962) and Van den Brenk (1963). Vos and Kaalen (1962) had demonstrated that DMSO protects human kidney cells in tissue culture, whereas other radioprotective compounds acting by the hypoxia mechanism had no effect in vitro. However, they can give no clear-cut explanation to account for the radioprotective action of DMSO. Additionally, Van den Brenk (1963) was unable to reduce the radioprotective action of DMSO in rats with high oxygen pressures. In view of these findings, Highman et al. (1967) propose that the radioprotective effect of DMSO is not due primarily to the hypoxia mechanism. However, they offer no alternative explanation.

Ashwood-Smith (1961) had observed that injection of DMSO resulted in a hypothermia in mice. This reaction was also reported following DMSO injection in rats (Highman et al., 1967). These findings were in accordance with those of Hope (1959) who reported a hypothermic effect in conjunction with other radioprotective substances in mice. However, it has been demonstrated by Bacq et al. (1965) that the hypothermia induced by antiradiation compounds was not linked to the radioprotective action.

In their report on the radioprotective effect of DMSO for rabbit muscle lactate dehydrogenase, Lohmann and his associates (1966) suggest that DMSO reacts with the $Fe^{+++}$ ion in the active site of the enzyme. This DMSO-enzyme complex would be responsible for the radioprotective effect observed.

Lappenbusch (1971) reported the effects of DMSO on the irradiation of more than 1,000 Holtzman rats. He observed that DMSO acted not only as a radioprotective agent in these rats, but that it could also act as a radiosensitizing compound as well, depending upon the dose of DMSO injected into the test animal.

Lappenbusch proposes that the primary mechanism of action of DMSO appears to be its effect upon the hematopoietic system following irradiation. He has demonstrated that injection of 7.5 g DMSO/kg, thirty minutes prior to exposure to 400R or 700R, prevented a drop in the number of red blood cells. This treatment also delays and reduces the fall in the number of white blood cells, reduced their period of low cell density, and afforded a rapid and complete replenishment of the white blood cell counts. The administration of DMSO also resulted in a limited polycythemia, which helped restore the initial drop in the peripheral red blood cell count after irradiation.

From these findings, Lappenbusch believes that the action of DMSO in affording radioprotection in rats lies

in its effect on the circulating blood cells. He does not, however, rule out the combined effects of hypoxia and enzyme-DMSO complex formation in addition to its effect on the hematopoietic system as the complete mechanism of action of DMSO.

In demonstrating that DMSO exerts a radioprotective effect in Drosophila, Mazar Barnett (1972) suggests yet an additional mechanism of action of DMSO in exerting this radioprotection. She points out that DMSO is capable of reacting with and changing the structure of polysaccharides, proteins, nucleic acids, and enzymes. Therefore, she feels that DMSO may act by reversibly altering the configuration of some of the more important macromolecules of the cell, making them less susceptible to radiation induced injury.

Rationale

It is evident from the descriptions of both EMS and DMSO that these two compounds exert a significant influence on the process of mutagenesis. EMS, an alkylating agent, has been established as a potent mutagen in a variety of organisms including Drosophila. The results of EMS action on DNA are often comparable to the effects of X-irradiation. DMSO has been established as an effective radioprotective substance in a number of organisms including Drosophila. Could DMSO act as an antimutagenic substance against EMS in Drosophila?

LeRoy (1972) has observed a complete X-linked recessive lethal mutation rate of 2.5% when a combination of EMS and DMSO were topically applied to adult male Drosophila. This frequency is significantly lower than the rate of 36.2% observed after feeding only EMS in the same experiments. Aschenbrener (1972) reported similar results when a reduction in the complete X-linked recessive lethal mutation frequency from 35.26% to 2.57% was observed following EMS feeding and combined EMS-DMSO application respectively.

The work of LeRoy (1972) and Aschenbrener (1972) is in contrast to the findings of Bhatia (1967). Working with Arabidopsis thaliana, Bhatia demonstrated an enhancing effect of 5% DMSO mixed with 10.0 µM EMS and applied to the growing point of eleven-day-old seedlings. DMSO increased the effect of EMS approximately two-fold in producing albino mutants. Bhatia believes that the enhancing effect is due to the carrier action of DMSO in transporting EMS into the growing cells faster and in larger quantities than EMS alone is capable of.

This study was undertaken in an attempt to determine if topical application of DMSO could inhibit the mutagenic action of EMS fed to Drosophila, and whether the sequence of administration of these two compounds (EMS followed by DMSO, DMSO followed by EMS) would cause a significant difference in the mutation frequencies.

# MATERIALS AND METHODS

Oregon-R (East Lansing) and Basc strains of Drosophila melanogaster were used in this study. All flies were reared and maintained on a cornmeal, agar, and molasses medium. The initial $P_1$ matings were made in quarter-pint bottles containing approximately 50 cc of medium, while further matings ($F_1$ and $F_2$) were made in eight dram shell vials containing 10 cc of culture medium. All cultures were maintained in constant light at a temperature of $25^{\circ} \pm 1^{\circ}$.

The chemicals tested in this study for mutagenic action and possible interaction were dimethyl sulfoxide (Fisher Scientific), and ethyl methanesulfonate (Eastman Organic).

Oregon-R males were treated with both DMSO and EMS. Five groups of flies were used: (1) Control--no treatment, (2) DMSO treatment only, (3) EMS treatment only, (4) DMSO treatment followed by EMS treatment, (5) EMS treatment followed by DMSO treatment.

The control group was treated with neither DMSO or EMS. Oregon-R males were simply fed a 1% sucrose solution for twenty-four hours and individually mated to 2-3 virgin Basc females.

The DMSO group was treated by dipping the abdomens of the flies into a drop of 100% DMSO for ten seconds. Etherized males were dipped into DMSO which had been deposited on the side of a 50 ml beaker by tilting the beaker to a $45^{\circ}$

angle, allowing a portion of the DMSO to remain on the side of the glass when the beaker was returned to an upright position. The flies were handled with a pair of watchmaker's forceps in such a way as to allow the underside of the abdomen to remain immersed in the drop of DMSO for ten seconds. Following dipping in DMSO, the flies were held in the air for a short period of time to allow some slight evaporation of the DMSO, and then placed in a paper lined culture bottle to recover from etherization. After one hour, these DMSO treated males were mated individually to 2-3 virgin Basc females.

EMS treatment of Oregon-R males followed the procedure of Lewis and Bacher (1968). Adult males were fed 0.025M EMS in a 1% sucrose solution. Approximately 10 ml of the EMS-sucrose solution was made available to the flies by saturating a plug of absorbent paper and placing it in the bottom of a quarter-pint culture bottle. Flies were placed in the bottle and allowed to feed on the EMS-sucrose solution for twenty-four hours. At the end of the feeding period, the males were immediately mated individually to 2-3 virgin Basc females.

The last two experimental groups were combinations of EMS and DMSO treatment. When these combined treatments were administered to the male flies, the individual EMS and DMSO treatment methods, described above, were followed. In these two groups the flies were allowed a one hour recovery period

between treatments.

In all five groups treated Oregon-R males were mated individually to virgin Basc females after treatment. These matings were made with one male and two or three females in each quarter-pint bottle. These flies were allowed to mate for periods of twenty-four hours or five days. Two different mating periods were used to determine the effect of the compounds on two separate spermatogenic stages (spermatozoa and spermatids). At the end of the mating period the males were removed and the females were permitted to lay eggs for three more days. After the egg laying period (four days and eight days respectively) all females were removed from the culture bottle.

$F_1$ X $F_1$ matings were made using one female and three or four males produced by the $P_1$ parents. In the event of a shortage of $F_1$ males, any male from a normal Basc strain was substituted.

Any $F_2$ culture not containing a lethal mutation will have four classes of progeny present. Half of the female progeny will be homozygous Basc females and the other half will be heterozygous Basc females. The male progeny will be composed of Basc males and wild-type males in equal numbers. Any $F_2$ culture which exhibits the complete absence of the wild-type male and has all of the other classes of progeny is scored as a culture containing an X-linked recessive lethal mutation.

Vials containing less than seven Basc males and no wild-type males were scored as presumptive lethals. Since the probability of producing six Basc males and no wild-type males is only 1/64, it is possible that this situation could arise in an $F_2$ culture in which there is really no lethal mutation. In this case several heterozygous $F_2$ virgin females were mated individually to Basc males. Again the absence of the wild-type male progeny in this $F_3$ generation will confirm the lethal.

## RESULTS

The results observed in this study are presented in such a manner that the data from the twenty-four hour mating group (Table 1) are considered independently of the five day mating group (Table 2). The results reported in these two tables were submitted to chi square analysis using the Yates correction factor where appropriate. It was found that there were no significant differences in the respective number of X-linked recessive lethal mutations induced by the compounds and combinations of compounds tested. Observing that the two different mating periods had no significant effect on the production of X-linked recessive lethal mutations (Table 3), the data obtained has been combined and presented in one additional table to facilitate presentation and evaluation (Table 4).

Table 1. Results of the Basc, X-linked recessive lethal mutation tests for treated male Drosophila allowed to mate for 24 hours.

| | Total $P_1$ matings | Fertile $P_1$ matings | Total $F_1$ matings | Fertile $F_1$ matings | Non lethal $F_2$ tests | Lethal $F_2$ tests | % lethal tests |
|---|---|---|---|---|---|---|---|
| Control | 20 | 14 | 587 | 574 | 574 | 0 | 0.0 |
| DMSO | 5 | 5 | 176 | 171 | 169 | 2 | 1.2 |
| EMS | 30 | 21 | 176 | 152 | 100 | 52 | 34.2 |
| DMSO-EMS | 30 | 19 | 145 | 124 | 86 | 38 | 30.6 |
| EMS-DMSO | 30 | 19 | 132 | 117 | 77 | 40 | 34.2 |

Table 2. Results of the Basc, X-linked recessive lethal mutation tests for treated male Drosophila allowed to mate for five days.

| | Total $P_1$ matings | Fertile $P_1$ matings | Total $F_1$ matings | Fertile $F_1$ matings | Non lethal $F_2$ tests | Lethal $F_2$ tests | % lethal tests |
|---|---|---|---|---|---|---|---|
| Control | 12 | 12 | 962 | 940 | 940 | 0 | 0.0 |
| DMSO | 6 | 6 | 558 | 534 | 527 | 7 | 1.3 |
| EMS | 120 | 77 | 619 | 502 | 319 | 183 | 36.5 |
| DMSO-EMS | 90 | 55 | 640 | 575 | 392 | 183 | 31.8 |
| EMS-DMSO | 90 | 54 | 512 | 460 | 298 | 162 | 35.2 |

Table 3. Chi square values for comparison of respective groups from 24 hour and 5 day mating period groups.

*Yates' correction used.

| 24 hour group | vs | 5 day group | Chi square value | Probability (approx.) |
|---|---|---|---|---|
| Control | | Control | 0.000 | 1.0 |
| DMSO | | DMSO | 0.060* | .80 |
| EMS | | EMS | 0.251 | .65 |
| DMSO-EMS | | DMSO-EMS | 0.066 | .80 |
| EMS-DMSO | | EMS-DMSO | 0.047 | .82 |

Table 4. Results of combined Basc, X-linked recessive lethal mutation tests for 24 hour mating group and five day mating group.

| | Total $P_1$ matings | Fertile $P_1$ matings | % Fertile $P_1$ matings | # $P_1$ matings producing lethals | % $P_1$ matings producing lethals | Total $F_1$ matings | Fertile $F_1$ matings | % fertile $F_1$ matings | Non lethal $F_2$ tests | Lethal $F_2$ tests | % lethal tests |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 32 | 26 | 81.3 | 0 | 0.0 | 1,549 | 1,514 | 97.7 | 1,514 | 0 | 0.0 |
| DMSO | 11 | 11 | 100.0 | 3 | 27.3 | 734 | 705 | 96.1 | 696 | 9 | 1.3 |
| EMS | 150 | 98 | 65.3 | 83 | 84.7 | 795 | 654 | 82.3 | 419 | 235 | 35.9 |
| DMSO-EMS | 120 | 74 | 61.7 | 63 | 85.1 | 785 | 699 | 89.0 | 478 | 221 | 31.6 |
| EMS-DMSO | 120 | 73 | 60.1 | 57 | 78.1 | 642 | 577 | 89.9 | 375 | 202 | 35.0 |

The control group was treated only by feeding a 1% sucrose solution to the flies for 24 hours. This resulted in an 81.3% fertility rate among the $P_1$ matings and a 97.7% fertility rate for the $F_1$ matings. The control group produced no lethal cultures in the $F_2$ generation establishing a 0.0% X-linked recessive lethal mutation rate as a background frequency (Table 4).

Drosophila treated by topically applying 100% DMSO to the abdomen produced a 100% fertility rate in the $P_1$ matings and a 96.1% fertility rate among the $F_1$ matings. Of the $P_1$ matings, 27.3% produced at least one X-linked recessive lethal mutation as scored in the $F_2$ generation. A total of 1.3% of all of the $F_2$ cultures in this group were scored as possessing an X-linked recessive lethal mutation (Table 4). Comparing this mutation frequency to that of the control group a chi square value with one degree of freedom of 16.0 was calculated (Table 5). This indicates that there is a highly significant increase in the X-linked recessive lethal mutation frequency as compared to the control group.

The EMS group was treated by feeding adult male Drosophila 0.025M EMS in a 1% sucrose solution for 24 hours. This treatment resulted in a 65.3% fertility in the $P_1$ matings and an 82.3% fertility frequency in the $F_1$ matings. Of the initial $P_1$ matings for this group 84.7% produced at least one X-linked recessive lethal mutation. An X-linked recessive lethal mutation frequency of 35.9% was computed

Table 5. Chi square values for comparison of data found in Table 4.

| | Control | DMSO | EMS | DMSO-EMS | EMS-DMSO |
|---|---|---|---|---|---|
| Control | -- | 16.0* | 606.0* | 528.0* | 583.0* |
| DMSO | 16.0* | -- | 275.0* | 340.0* | 260.0* |
| EMS | 606.0* | 275.0* | -- | 2.82 | 0.11 |
| DMSO-EMS | 528.0* | 234.0* | 2.82 | -- | 1.63 |
| EMS-DMSO | 583.0* | 260.0* | 0.11 | 1.63 | -- |

* Yates' correction used.

p = .05 for value of 3.84.
p = .01 for value of 6.63.

for this group after scoring the $F_2$ progeny (Table 4). This mutation rate was found to be significantly greater than the X-linked recessive lethal mutation rates of the control group and the DMSO treated group when submitted to chi square analysis (Table 5).

The DMSO-EMS group was treated in two ways. First the flies were dipped into 100% DMSO. These DMSO treated flies were then allowed to feed on 0.025M EMS in a 1% sucrose solution for 24 hours. This particular treatment resulted in a 61.7% fertility in the $P_1$ matings and an 89.0% fertility rate for the $F_1$ matings. Among these $P_1$ matings, 85.1% resulted in at least one X-linked recessive lethal mutation as scored in the $F_2$ generation. This combination of chemical treatments resulted in an X-linked recessive lethal mutation frequency of 31.6% (Table 4). This is lower than the mutation rate produced by EMS alone, but chi square analysis provides a value of 2.82 which demonstrates that the reduction is only significant at the .08 level. However, when the mutation rate of the DMSO treated group is statistically compared to that of the DMSO-EMS group a chi square value of 234.0 is computed. This establishes a highly significant increase in the X-linked recessive lethal mutation frequency when the DMSO treatment is compared to the more mutagenic DMSO-EMS treatment.

The EMS-DMSO group was treated in the same manner as the DMSO-EMS group except that the sequence of the treatments

was reversed. Treatment of adult male Drosophila with 0.025M EMS and then 100% DMSO resulted in a 60.1% fertility among the $P_1$ matings and an 89.9% fertility rate for the $F_1$ matings. Of the $P_1$ matings, 78.1% resulted in the production of at least one X-linked recessive lethal mutation. After scoring all of the $F_2$ cultures an X-linked recessive lethal mutation rate of 35.0% was computed (Table 4). When this mutation rate is compared by chi square analysis to the mutation rates of the EMS and DMSO-EMS groups, no significant difference is observed (Table 5). A significant difference is obtained when the EMS-DMSO mutation frequency of 35.0% is compared to the 1.3% mutation rate produced by DMSO alone (Table 5).

## DISCUSSION

The major objective of this study was to determine the effect of topically applied DMSO on the mutagenicity of EMS ingestion in Drosophila. To determine the effects of these two chemicals on adult male Drosophila, a series of Basc X-linked recessive lethal mutation experiments were carried out. This particular technique measures only the production of X-linked recessive lethal mutations in treated adult flies. Experiments were carried out on Drosophila which were allowed to mate for periods of 24 hours or five days after treatment of the males.

The results obtained from these two mating period groups are presented in Tables 1 and 2. DMSO produced an X-linked recessive lethal mutation rate of 1.2% in the 24 hour group and 1.3% in the five day group. Chi square analysis of these two frequencies using the Yates' correction provides a value of 0.06. This indicates that there is no significant difference in the frequency of X-linked recessive lethal mutations produced when Drosophila are allowed to mate for 24 hours or five days after treatment with DMSO. This finding holds true for all five groups when the mutation rates after each treatment are statistically compared (Table 3). These results are in agreement with the findings of Fahmy and Fahmy (1957) who have reported that EMS has its greatest effect on the later stages of spermatogenesis. These include the post-meiotic stages, spermatozoa and spermatids. Martin (1965) has reported that during the first seven days after treatment, the sperm ejaculated by Drosophila were either spermatozoa or spermatids at the time of treatment. It is obvious from these reports and this study that ejaculations in the first 24 hours after treatment and the first five days after treatment both contain sperm which have been preferentially acted upon by the chemicals being tested. It can therefore be stated that there is no significant difference in the production of X-linked recessive lethal mutations for the two mating periods studied in this experiment.

From this point on, all results which are discussed

are compiled in Table 4 and represent data from the 24 hour and five day mating groups.

No X-linked recessive lethal mutations were observed in the 1,514 chromosomes tested in the control group. Wallace (1970) reports a spontaneous mutation rate to X-linked recessive lethals for a variety of wild-type males at 0.24%. The chi square value between the 0.0% mutation frequency observed in this study and the 0.24% mutation frequency observed by Wallace is 2.65. This chi square value indicates that the mutation rate observed for the control group in this experiment does not differ significantly from the spontaneous rate for wild-type males reported by Wallace.

LeRoy (1972) observed a 0.27% X-linked recessive lethal mutation frequency for the nontreated control group in his work. Similarly, Aschenbrener (1972) reported a mutation rate of 0.26% for his control group in a corresponding study. Chi square analysis of these two mutation rates with the X-linked recessive lethal mutation rate of the control group in this study using Yates correction indicates a significant difference was observed. The higher mutation rate was significant in the latter two cases when compared to the present study but not in the comparison with the data Wallace reports because of the number of chromosomes tested.

Alexander (1966) reported a 0.0% X-linked recessive lethal mutation frequency for 1097 chromosomes tested as a control when her Drosophila were allowed to mate for six days.

The mutation rate of the control group of this study produced a 0.0% X-linked recessive lethal mutation frequency after flies were allowed to mate for five days. Therefore, the control group in this work demonstrated no deviation from Alexander's results in the spontaneous production of X-linked recessive lethal mutations.

Dipping Drosophila into 100% DMSO for ten seconds resulted in an X-linked recessive lethal mutation rate of 1.3% in this study. This value is significantly higher than the 0.0% mutation rate observed in the control group and is also significantly higher than the 0.0% X-linked recessive lethal mutation rates observed by LeRoy (1972) and Aschenbrener (1972), after DMSO dipping. LeRoy and Aschenbrener both used 1.0M DMSO for their dipping experiments, while 100% DMSO was used in this study. The higher concentration of DMSO used in this study was probably more effective in inducing genetic damage than was the 1.0M DMSO used by the previous two workers, therefore, a higher X-linked recessive lethal mutation frequency was observed.

Alexander (1966) reported a 0.28% X-linked recessive lethal mutation rate after treatment with DMSO and allowing a six day mating period. The mutation rate of 1.3% found in this experiment is significantly higher than that of Alexander. Alexander used concentrated DMSO in her work but she does not state a time for the duration of the DMSO treatment. In my work I used 100% DMSO and dipped the flies for

ten seconds. This amount of time for direct exposure to the 100% DMSO could account for the significant rise in the mutation frequency.

Several researchers have stated that DMSO is capable of forming complexes with a variety of chemical compounds. Lohmann et al. (1966) suggest that DMSO forms a complex with the $Fe^{+++}$ ion in rabbit muscle lactate dehydrogenase, Mazar Barnett (1972) states that DMSO can react with and change the structure of polysaccharides, proteins, nucleic acids, and enzymes.

The present research has shown that DMSO is somewhat mutagenic in Drosophila. This action could take place through the reaction of DMSO with the nuclear DNA itself or with some DNA associated compound such as a nuclear protein. This reaction could result in stress on the DNA structure or actual damage to the molecule, inducing a mutation. Further work is needed to determine the actual mechanism of DMSO in producing mutations.

EMS has been previously reported to be a potent chemical mutagen, capable of producing mutation frequencies ranging from 18.3% with $1.16 \times 10^{-2}$M EMS (Fahmy and Fahmy, 1957) to 70% with $3.12 \times 10^{-2}$M EMS (Alderson, 1965). Experiments in this study resulted in a 35.9% X-linked recessive lethal mutation frequency, which is in agreement for EMS induced mutation rates reported by a variety of previous workers (Fahmy and Fahmy, 1957; Alderson, 1965; Kahn, 1969; Sega and

Lee, 1970; Myszewski, personal communication). Comparison of the 35.9% EMS induced X-linked recessive lethal mutation frequency with the mutation frequencies of the control group and the DMSO treated group demonstrates that a significantly greater proportion of X-linked recessive lethals was produced by the EMS treatment.

The DMSO-EMS group was treated by topical application of 100% DMSO for ten seconds followed by allowing these DMSO treated males to feed on 0.025M EMS in a 1% sucrose solution for 24 hours. Mazar Barnett (1972) reported that DMSO treatment was capable of protecting Drosophila against radiation induced mutation. It was thought that in this study, application of DMSO might protect against the mutagenic effects of EMS. After pre-treatment of Drosophila with 100% DMSO for ten seconds, flies treated with 0.025M EMS produced an X-linked recessive lethal mutation frequency of 31.6%. This mutation rate of 31.6% is lower than the 35.9% mutation rate reported after only EMS treatment. However, submitting the two frequencies to chi square analysis results in a value of 2.82. This chi square value indicates that the reduction in the mutation frequency following DMSO pre-treatment is only significant at the 10% level, and therefore cannot be interpreted as proof that DMSO can reduce the mutagenic action of EMS in Drosophila.

The reduced mutation frequency observed in the DMSO-EMS group could be attributed to the actual amount of EMS

ingested by the pre-treated males, reduction in the fertility of the DMSO treated males, or the production of a greater amount of dominant lethals. LeRoy (1972) reported that only 52.3% of the eggs layed by females inseminated by DMSO treated males proceeded to hatching, indicating that DMSO treatment produced a 47.7% dominant lethal mutation frequency. The induction of dominant lethal mutations may be masking many of the X-linked recessive lethal mutations produced by DMSO-EMS treatment in this study.

Aschenbrener (1972) has demonstrated that adult male Drosophila topically treated with a mixture of DMSO and EMS produced an X-linked recessive lethal mutation rate of 2.57%. LeRoy (1972) similarly reported a 2.50% X-linked recessive lethal mutation frequency following topical application of DMSO and EMS in one solution. Both of these mutation rates are significantly lower than the frequency of 31.6% reported in this study for the DMSO-EMS group. The discrepancy between these values appears to reside in the method of treatment. In this experiment, DMSO was topically applied to the abdomen of adult male flies. Aschenbrener and LeRoy prepared a mixture of the DMSO and EMS solutions, and then applied this mixture to the flies. There appears to be two possible reasons for the reduced mutation rate. First, in mixing the two chemicals, a neutralizing effect may have occurred. It seems that the DMSO has neutralized a majority of the reactive alkyl groups on the EMS molecule, therefore, reducing its

mutagenicity. However, Bhatia (1967) has demonstrated an enhancing effect of 5% DMSO mixed with 10.0M EMS when applied to the growing areas of Arabidopsis. In Bhatia's results the DMSO has not neutralized the EMS, but has increased its mutagenicity twofold. The findings of Bhatia appear to discount the idea that DMSO is capable of neutralizing EMS.

The second possibility that could account for the reduced mutagenicity of a DMSO-EMS mixture is the fact that not enough EMS is getting to the testes of the fly. Drosophila have a chitinous exoskeleton and this may result in a partial barrier in the path of the DMSO-EMS solution. It is apparent from the 1.3% X-linked recessive lethal mutation rate observed in this experiment after DMSO treatment, and the 47.7% dominant lethal mutation frequency observed by LeRoy (1972) after DMSO application, that DMSO is capable of entering the testes of Drosophila when applied to the abdomen of the fly. However, EMS may reduce the penetrating ability of DMSO and therefore reduce the amount of the DMSO-EMS solution that actually reaches the testes. This would result in a reduced mutation rate.

Bhatia (1967) believes that the enhancing effect of DMSO on EMS is due to the increased permeability of the mixture over that of EMS alone. However, Bhatia used only a 5% DMSO solution in his mixture and the mixture only had to penetrate the cell wall and cell membrane of the Arabidopsis seedling, a herbaceous plant.

The EMS-DMSO group was treated in a manner similar to the DMSO-EMS group except that the sequence of treatments was reversed. In this study an X-linked recessive lethal mutation rate of 35.0% was observed. Chi square analysis showed that this frequency was not significantly different from the 35.9% rate after EMS treatment or the 31.6% rate observed after DMSO-EMS treatment. It has been reported by Ashwood-Smith (1961) that DMSO administration after irradiation resulted in no protective effect in mice as it had when administered before irradiation. This fact can also be observed in this study. When DMSO was applied after EMS treatment, the mutation rate dropped only 0.9%. This drop is probably due to experimental error and is not significant.

In observing the fertility of the $P_1$ matings it can be seen that topical application of 100% DMSO has a definite effect in reducing the reproductive capabilities of the $P_1$ parents when applied in conjunction with EMS. EMS treatment alone resulted in a 65.3% $P_1$ fertility. Application of DMSO in addition to EMS treatment reduced the $P_1$ fertility even more to 61.7% and 60.1% for the two combined treatment groups. Chi square analysis demonstrates that the $P_1$ fertility frequencies for the EMS, DMSO-EMS, and EMS-DMSO groups are not significantly lower than the $P_1$ fertility for the control group. However, the $P_1$ fertilities for EMS, DMSO-EMS, and EMS-DMSO are significantly lower than the 100% $P_1$ fertility of the DMSO group. This reduction in fertility

could be due to sterilizing effect of DMSO application to EMS treated males. The EMS itself appears to have some sterilizing properties. The reduction in fertility could also be attributed to the refusal of females to mate with recently treated males. A reduced fertility after DMSO application has previously been reported by LeRoy (1972) and Aschenbrener (1972). They attribute this reduction to the induction of dominant lethal mutations by DMSO.

In observing the proportion of $P_1$ matings which produced X-linked recessive lethal mutations it is evident that all groups which were treated with EMS at some time produced a high frequency of lethality in the individual males treated. After EMS treatment alone, 84.7% of the males treated produced at least one X-linked recessive lethal mutation. Similarly, the DMSO-EMS group showed 85.1% of the $P_1$ matings producing at least one lethal $F_2$ culture. The EMS-DMSO group had a lower frequency of $P_1$ matings producing at least one lethal but the reduction was not significant when compared to the EMS and DMSO-EMS groups.

## CONCLUSION

The major objective of this study was to determine if topically applied 100% DMSO could act as an antimutagen in reducing the amount of X-linked recessive lethal mutations produced by the alkylating agent EMS. It has been reported

that DMSO can act as a radioprotective substance in protecting a variety of organisms against the mutagenic damage induced by irradiation. Alkylating agents often produce mutations similar to those produced by irradiation and are referred to as "radiomimetic substances."

The results of this study show that pretreatment of Drosophila with topical application of 100% DMSO did not significantly reduce the X-linked recessive lethal mutation rate after feeding 0.025M EMS for 24 hours. Similarly, treatment of Drosophila with 100% DMSO after EMS treatment did not significantly reduce the X-linked recessive lethal mutation frequency. Therefore, DMSO does not act as an antimutagen against EMS in Drosophila under the conditions of this study.

It was found that the topical application of 100% DMSO to the abdomens of adult male Drosophila did significantly increase the frequency of X-linked recessive lethal mutations. Therefore, 100% DMSO appears to be slightly mutagenic in Drosophila producing a mutation frequency of 1.3%. It also appears that the topical application of DMSO reduces the overall reproductive capabilities of adult male Drosophila.

Further study into the effects of these two chemicals on Drosophila is necessary at this time. Microinjection of DMSO into the abdomens of the flies rather than topical application may allow more DMSO to be absorbed by the organism and

thus exert a greater influence on the production of X-linked recessive lethal mutations induced by EMS. It is necessary to determine the amount of topically applied DMSO actually does make its way to the testes.

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